

New

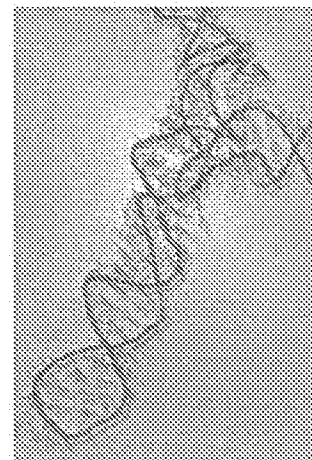
Custom and library siRNA for efficient gene silencing

High-quality short interfering RNA (siRNA) from QIAGEN allows efficient gene silencing in eukaryotic cell culture by targeting complementary endogenous mRNA. This effect, termed RNA interference (RNAi), enables targeted gene silencing in functional genomics studies. Now, QIAGEN offers a custom siRNA oligonucleotide synthesis service that includes a range of modifications and also library siRNAs directed against common target genes.

QIAGEN siRNA Oligo Synthesis Service offers:

- ◆ High-purity siRNA for efficient gene silencing
- ◆ Custom and library siRNA ready to use in cell transfection
- ◆ Expert advice on siRNA design
- ◆ Labeled siRNA

►► Custom and library siRNA, page 24



New

Multiplex PCR that simply works — the new QIAGEN® Multiplex PCR Kit

The new QIAGEN® Multiplex PCR Kit is the first kit specifically developed for multiplex PCR. The simple multiplex master-mix solution eliminates the need for lengthy optimization procedures, such as adjusting the amounts of Mg^{2+} and enzyme or even, as frequently required, adjusting primer concentrations. Now standard multiplex PCR applications are fast and easy to perform.

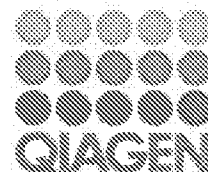
Advantages of the new QIAGEN Multiplex PCR Kit:

- ◆ No optimization required — easy assay development with simple master mix

- ◆ High specificity and sensitivity — stringent hot start with HotStarTaq® DNA Polymerase and increased sensitivity with the unique new multiplex PCR buffer
- ◆ Versatile for many applications — including typing of transgenic animals and plants, detection of bacteria and viruses, and microsatellite analyses

- ◆ Easy to use and cost-effective — simple reaction setup for fast and reproducible results

►► New QIAGEN Multiplex PCR Kit, page 14



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No optimization required

Establishing multiplex PCR assays is a tedious and time-consuming procedure. In most cases, not all PCR products are generated, and nonspecific background products, such as primer-dimers, are often observed. To get acceptable results requires extensive optimization of the amounts of Taq DNA polymerase, Mg^{2+} , additional reagents, and primers. Often, cycling parameters or reaction buffer formulations need to be changed. In many cases, the results are still disappointing, requiring further extensive optimization (see flowchart).

The QIAGEN Multiplex PCR Kit eliminates the need for optimization, making the development of multiplex PCR assays both simple and fast. The master mix contains pre-optimized concentrations of HotStarTaq DNA Polymerase and $MgCl_2$, plus dNTPs and a PCR buffer newly developed for multiplex reactions. Excellent results can be achieved the first time using fixed primer concentrations and the standard multiplex PCR cycling protocol, included in the QIAGEN Multiplex PCR

Handbook. Typically, there is no need to adjust reaction parameters (see flowchart).

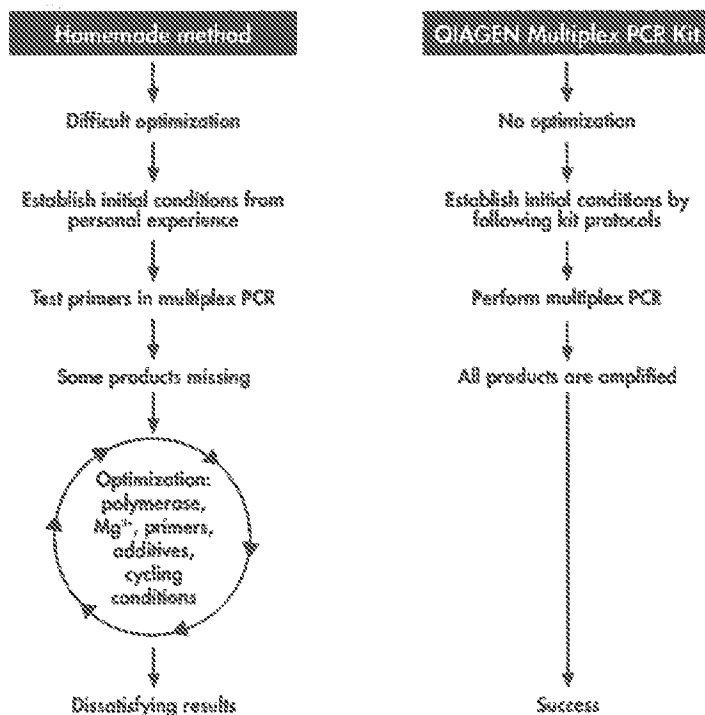
High specificity and sensitivity

Nonspecific PCR products and primer-dimers are often generated during multiplex PCR due to the large amount of different primers in the reaction. Amplification of these nonspecific products competes with amplification of the desired PCR products, leading to decreased sensitivity.

With the QIAGEN Multiplex PCR Kit, specificity is significantly increased by use of HotStarTaq DNA Polymerase in the master mix. This enzyme enables a highly stringent hot start and allows reaction setup at room temperature without nonspecific artifacts.

The newly developed multiplex PCR buffer in the master mix further enhances the sensitivity of the multiplex reaction. This innovative, new formulation was specifically developed and optimized for multiplex PCR. In contrast to conventional PCR reagents, the new buffer contains a balanced combination of salts and additives to ensure comparable efficiencies for annealing and extension of all primers in the reaction (Figure 1).

Multiplex PCR Setup



Successful 16plex PCR Using the QIAGEN Multiplex PCR Kit

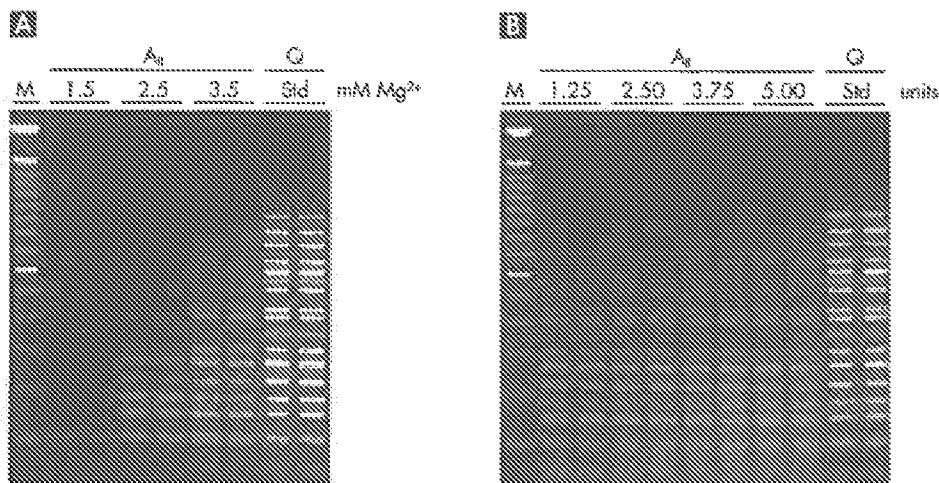


Figure 1 Multiplex PCR of 16 targets (99–955 bp) was carried out for 35 cycles using standard conditions (Std) for the QIAGEN Multiplex PCR Kit (Q) without further optimization or using a variety of conditions with a hot-start DNA polymerase from Supplier A₁ (A₁). **A** Comparison using 2.5 units/50 µl reaction of the hot-start DNA polymerase from Supplier A₁ and with the indicated Mg²⁺ concentrations. **B** Comparison using the optimized Mg²⁺ concentration (3.5 mM) for the hot-start DNA polymerase from Supplier A₁ and the indicated amounts of enzyme per 50 µl reaction. M: markers.

Versatile for many applications

The QIAGEN Multiplex PCR Kit is well suited for many types of multiplex PCR applications using different detection methods. PCR products can be visualized on agarose gels or detected using fluorescently labeled primers. Detection of fluorescently labeled products can be performed on automatic gel-based DNA sequencers (e.g., the ABI PRISM® 377 DNA Sequencer; Figure 2), as well as those based on capillary

electrophoresis (e.g., the ABI PRISM 310 Genetic Analyzer).

The QIAGEN Multiplex PCR Kit has been successfully used for typing and analysis of transgenic animals and plants, amplification of multiple DNA regions for SNP analysis, and typing and detection of bacteria and viruses. A special protocol for amplification and analysis of microsatellites is also provided. In addition, the kit contains a protocol ►

Microsatellite Analysis with Optimized QIAGEN Protocol

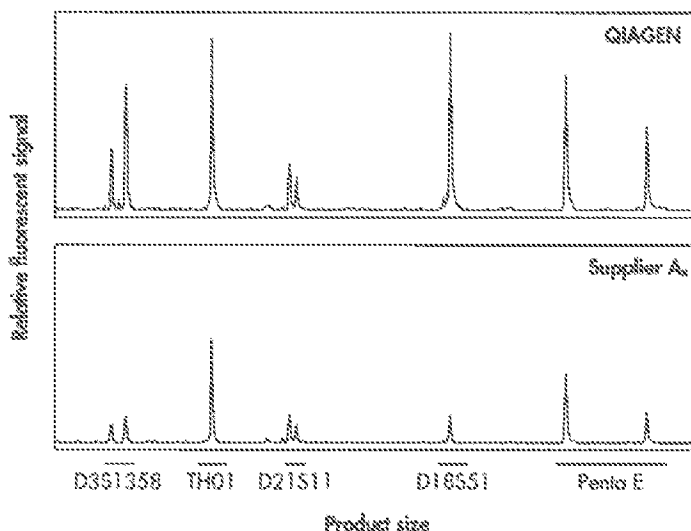


Figure 2 Analysis of microsatellite loci D3S1358, TH01, D21S11, D18S51, and Penta E was carried out using 1 ng of K562 human genomic DNA and fluorescein-labeled primers. Reactions were analyzed on the ABI PRISM 377 Sequencer. Top: High sensitivity and uniform signal intensity using the QIAGEN Multiplex PCR Kit and the microsatellite protocol included in the QIAGEN Multiplex PCR Handbook. Bottom: Results using a hot-start DNA polymerase from Supplier A₁.

for multiplex amplification using Q-Solution, suitable for targets with high GC content or complex secondary structure.

Easy to use

The QIAGEN Multiplex PCR Kit contains a master mix specifically designed for multiplex PCR applications. The master-mix format makes handling and reaction setup both fast and easy. Use of a master-mix format reduces time and handling for reaction setup and increases reproducibility by eliminating many possible sources of pipetting errors. The master mix is stable at -20°C and 4°C for many months. Storage at 4°C eliminates thawing time, providing even faster setup of

multiplex PCR assays. Reaction setup at room temperature also makes the QIAGEN Multiplex PCR Kit the ideal tool for automated PCR setup.

The easy-to-follow protocol and the robust universal multiplex cycling conditions give you reliable results faster. This is especially important for simultaneous analysis of multiple samples, such as for genotyping transgenic animals and plants (Figure 3).

Fast and easy assay development with increased reproducibility makes the QIAGEN Multiplex PCR Kit a cost-effective and timesaving solution for multiplex PCR applications. ■

Genotyping Transgenic Mice

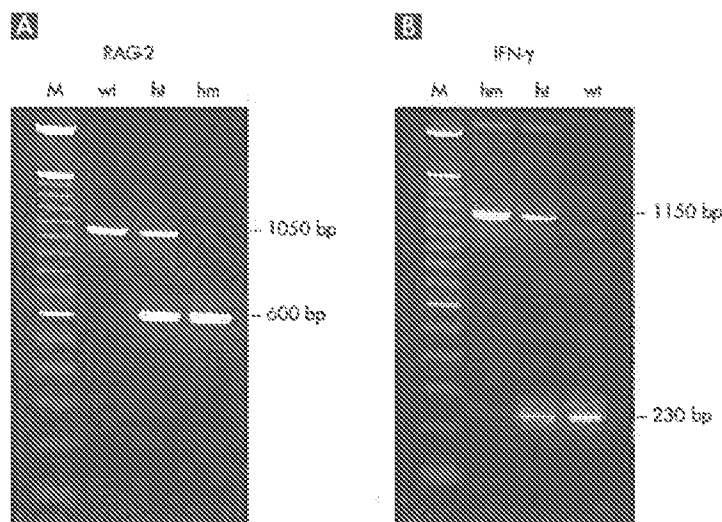
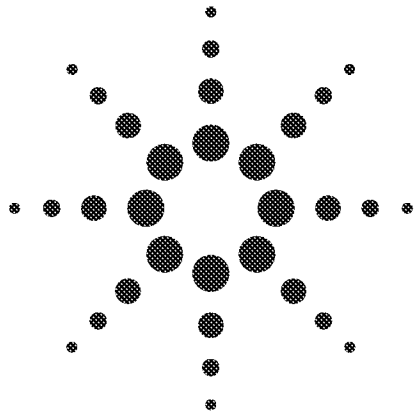


Figure 3 Transgenic mice were screened using the QIAGEN Multiplex PCR Kit and sets of 3 primers to distinguish wild-type (wt), heterozygous mutant (ht), and homozygous mutant (hm) mice. **A** Using a primer set for the recombination activating gene 2 locus. **B** Using a primer set for the interferon-γ gene locus. M: markers. [Data kindly provided by S. zur Lage and S. Weiss, National Research Center for Biotechnology, Braunschweig, Germany.]

Ordering Information

Product	Contents	Cat. No.
QIAGEN Multiplex PCR Kit — for fast and efficient multiplex PCR		
QIAGEN Multiplex PCR Kit (100)	For 100 multiplex PCR reactions: 2x QIAGEN Multiplex PCR Master Mix (containing 6 mM MgCl ₂ , 3 x 0.85 ml), 5x Q-Solution (1 x 2.0 ml), distilled water (2 x 1.7 ml)	206143



Highly efficient multiplex PCR using novel reaction chemistry

Application

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Abstract

A new multiplex PCR kit has been introduced that allows amplification of multiple gene targets without the need to optimize PCR conditions. In this Application Note, the kit was used to amplify up to 19 targets in a single PCR step under standard conditions. Amount and purity of the PCR products has been confirmed using the Agilent 2100 bioanalyzer.



Introduction

Multiplex PCR is a powerful technique enabling amplification of two or more products in a single reaction. Typically, it is used for genotyping applications where simultaneous analysis of multiple markers is required, such as typing of normal and genetically modified animals and plants, detection of pathogens or genetically modified organisms (GMOs), or for microsatellite analyses. Multiplex assays can be tedious and time-consuming to establish, requiring lengthy optimization procedures, such as adjusting primer concentrations, Mg^{2+} concentration, and the amount of enzyme. In many cases, the results are still disappointing and further extensive optimization may be required. However, due to novel developments in the reaction chemistry, multiplex assays are now simple and straightforward to establish. The combined use of a highly stringent hot start with a unique PCR buffer specially developed for multiplex reactions, makes the QIAGEN® Multiplex PCR Kit highly suited for multiplex PCR applications. The newly developed reaction buffer, containing a special multiplex PCR-enhancing synthetic factor, eliminates the need for optimization — even when using equimolar primer concentrations. In this study, we describe the effects of different reaction parameters and how the QIAGEN Multiplex PCR Kit minimizes the need for optimization of multiplex PCR assays. The results were visualized using the Agilent 2100 bioanalyzer.

Material and methods

Genomic DNA was isolated from human K562 cells using the DNeasy® Tissue Kit and 20 ng were used as template for multiplex PCR. PCR was performed using either QIAGEN Multiplex PCR Master Mix (containing HotStarTaq® DNA Polymerase), or using standard Taq DNA polymerase in an otherwise identical reaction mixture, or using a Taq DNA polymerase with antibody-mediated hot start and KCl reaction buffer from Supplier L. Equal volumes of each multiplex PCR were analyzed on the Agilent 2100 bioanalyzer using the DNA 1000 LabChip® kit according to the supplied instructions.

Results and discussion

Effect of hot start on multiplex PCR specificity

Typically, a primer concentration of 0.2–0.5 μM is used in conventional PCR. In contrast, the total primer concentration in multiplex PCR can be as high as 2–4 μM , depending on the number of different primer pairs in the reaction. The large number of primers often results in the generation of non-specific PCR products and primer-dimers, reducing the specificity and sensitivity of the multiplex PCR. Using a stringent hot start to increase PCR specificity can prevent the generation of these non-specific products. Figure 1 shows a comparison of hot-start multi-

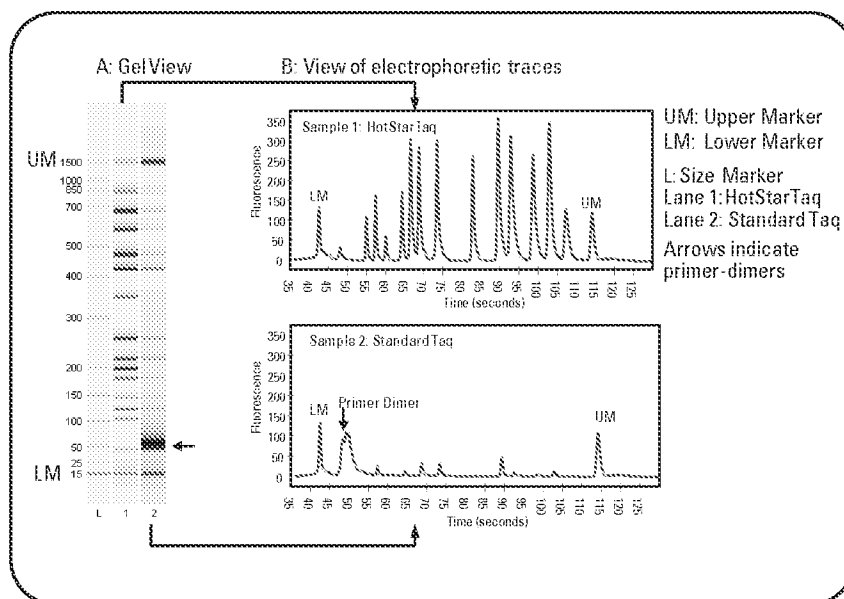


Figure 1
Effect of Hot Start on Multiplex PCR. Multiplex PCR of 13 targets (99–955 bp) was carried out for 35 cycles in a 50 μl reaction volume using the standard protocol in the QIAGEN Multiplex PCR handbook. Multiplex reactions were carried out using QIAGEN Multiplex PCR Master Mix (containing HotStarTaq DNA Polymerase) (QIAGEN Multiplex PCR Kit) or standard Taq DNA Polymerase (Taq). Equal volumes of each multiplex PCR were analyzed on the Agilent 2100 bioanalyzer using the DNA 1000 LabChip kit. A. Gel-like image of collected data. B. Electrophoretic trace of multiplex PCR.

plex PCR, using the QIAGEN Multiplex PCR Kit, and multiplex PCR, using a standard Taq DNA polymerase without a hot start. Analysis was carried out on the Agilent 2100 bioanalyzer using the DNA 1000 LabChip kit. Use of standard Taq DNA polymerase results in the generation of large amounts of primer-dimers and low efficiency of amplification. In contrast, the stringent hot start provided by HotStarTaq DNA Polymerase in the QIAGEN Multiplex PCR Kit leads to reproducible and efficient amplification of all 13 PCR products without generating nonspecific artifacts.

Effect of novel multiplex PCR buffer chemistry on primer annealing

With multiplex PCR, the annealing efficiencies of the different primers in the reaction are usually dissimilar. Although it is possible to design primers that have a similar annealing temperature, the T_m of each primer does not always provide a good indication of its annealing efficiency. In addition, other factors such as the 3'-end sequence of primers may affect the efficiency of primer extension by Taq DNA polymerase. Efficient primer annealing and extension, irrespective of primer sequence, is achieved by a special synthetic factor in QIAGEN Multiplex PCR Master Mix. This synthetic factor increases the local concentration of primers at the template DNA and stabilizes specifically bound primers (figure 2). In addition, nonspecific primer binding is avoided by a balanced combination of salts and the specially optimized NH_4^+ concentration in the QIAGEN Multiplex PCR Master Mix. NH_4^+ , which exists

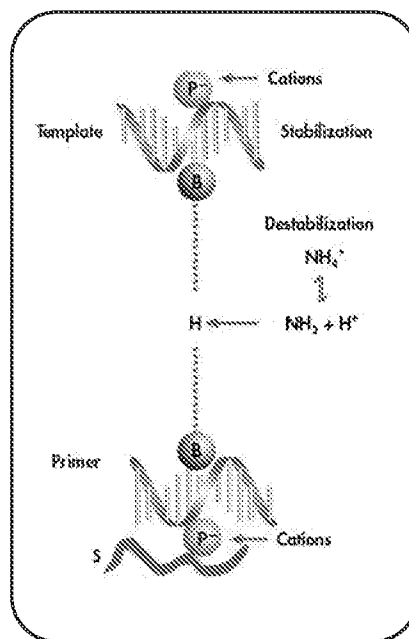


Figure 2

Effect of Novel Synthetic Factor and NH_4^+ Ions on Primer Annealing. Mg^{2+} and other salts bind to phosphate groups (P) on the DNA backbone, which stabilizes the annealing of the primers to the template. NH_4^+ , which exists both as the ammonium ion and as ammonia under thermal-cycling conditions, can interact with the hydrogen bonds between the bases, destabilizing principally the weak hydrogen bonds at mismatched bases (B) of non-specifically bound primers. The synthetic factor (S) pushes the primers toward the template and stabilizes specifically bound primers, enabling efficient extension of all primers in the reaction.

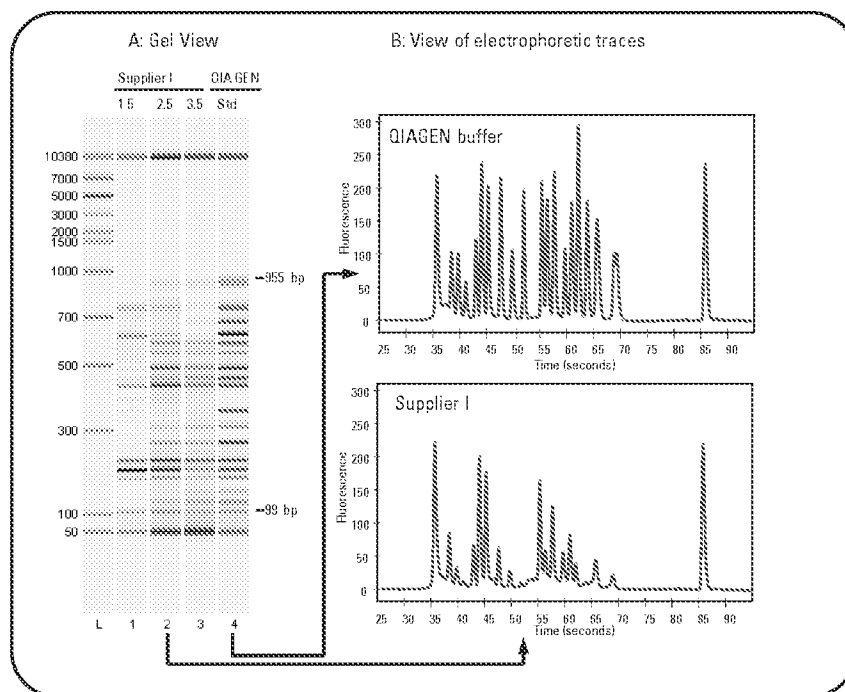


Figure 3

Effect of PCR Buffer on Multiplex PCR. Multiplex PCR of 19 targets (99–955 bp) was carried out for 35 cycles using standard conditions (Std) for the QIAGEN Multiplex PCR kit without optimization, or using the indicated Mg^{2+} concentrations with a hot-start enzyme and supplied KCl-based buffer from Supplier I. Equal volumes of each multiplex PCR were analyzed on the Agilent 2100 bioanalyzer using the DNA 7500 LabChip kit. A. Gel-like image of collected data. B. Electrophoretic trace of multiplex PCR.

predominantly as ammonia (NH₃) under thermal-cycling conditions, interacts with the relatively weak hydrogen bonds formed when primers bind nonspecifically to the template DNA and destabilizes these nonspecifically bound primers. Figure 3 shows that in contrast to conventional PCR reagents, the formulation of the new QIAGEN Multiplex PCR Master Mix, ensures comparable efficiencies for annealing and extension of all primers in the reaction without further optimization.

Conclusion

Establishing multiplex PCR assays is easy and fast using the QIAGEN Multiplex PCR Kit. Tedious optimization procedures are virtually eliminated. These properties make the kit well suited for multiplex applications, including genotyping of transgenic organisms, detection of pathogens or GMOs, and microsatellite genotyping (e.g., short tandem repeat (STR) and variable number tandem repeat (VNTR) analyses). The stringent hot start provided by HotStarTaq DNA Polymerase eliminates non-specific PCR products and primer-dimer formation in multiplex PCR, while a novel synthetic factor in the multiplex PCR buffer allows efficient annealing of multiple primers under identical cycling conditions. Visualization of multiplex PCR products can be performed efficiently using the Agilent 2100 bioanalyzer and DNA LabChip kits, because of the accurate sizing and quantitation that can be achieved.

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EXHIBIT C

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Overview and Research Interests

Dirk Löffert, Ph.D., is Vice President Research & Development and Head of QIAGEN Sample & Assay Platform Technologies Global R&D. He joined QIAGEN in 1996. His responsibilities include technology and product development for sample preparation and assay solutions for the Life Sciences market including applications such as epigenetics analysis, genotyping and gene expression analysis. This comprises product development but also development of core technologies for sample collection, stabilization and biomolecule isolation and assay technologies such as Pyrosequencing, PCR and real-time PCR and isothermal global and target-specific amplification technologies.

Education

Ph.D. in Molecular Biology and Immunology from the Institute for Genetics at the University of Cologne, Germany.

Selected Publications

Lowe B, O'Neil D, Loeffert D, Nazarenko I., "Distribution of Human papillomavirus load in clinical specimens," *J Virol Methods* 2011 Apr;173(1):150-2.

Thai H, Rangwala S, Gay T, Keating K, McLeod S, Nazarenko I, O'Neil D, Pfister D, Loeffert D., "An HPV 16, 18, and 45 genotyping test based on Hybrid Capture technology," *J Clin Virol* 2009 Jul; 45 Suppl 1:S93-7.

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